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D-7672 /linuron sc

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

CASWELL#528

DATE: MAY 16 1980

SUBJECT: EPA Reg.#352-270; Lorox; Long-term Feeding Study in Rats with 3-(3,4-dichlorophenyl-1-methoxy-1-methylurea (Lorox, Linuron, INZ-326) Acc#241897-8

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Recommendations:

1) Linuron produced statistically significant dose-related increases of benign testicular interestitial cell adenomas in male rats fed 125 and 625 ppm. This effect triggers an oncogenic RPAR criterion. Toxicology Branch is, with this memo, referring Linuron to SPRD for pre-RPAR review.

Review:

1) Long-term feeding study in rats with 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (Lorox, Linuron: INZ-326) Medical Research Project No. 2629; Haskell Laboratory Report No. 100-80; Final Report.

Test Material: INZ-326-118; N.B. 7673-8; 97% A.I., Linuron

Three hundred and fifty-two male and 352 female weanling ChR-CD rats were housed in pairs, sexes separate, in stainless-steel, wire-mesh cages. During a pretest period of one week, they were given ground Purina Laboratory Chow (GPLC) and observed with respect to weight gain, eating habits and the presence of any clinical signs of diseases. At the end of this period, 320 rats of each sex were selected on the basis of weight gain and freedom from gross respiratory disorders or other clinical signs of disease and were divided into four groups of 80 males and four groups of 80 females. The assignment of animals to groups by computer generated random numbers was such that the mean weight within each group was approximately the same. Rats were toe-clipped for individual identification. Groups were randomly assigned to receive the following diets ad libitum:

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Group		
Male	Female	Diet
I	II	GPLC (control)
III	IV	GPLC + 50 ppm
V	VI	GPLC + 125 ppm
VII	VIII	GPLC + 625 ppm

Diets were prepared fresh each week and stored under refrigeration until used. All animals were weighed once a week during the first six months of the study, biweekly for the next six months and monthly for the duration of the study.

During the test period, rats were examined daily for abnormal behavior or clinical signs of toxicity.

Food consumption was determined on a group basis at each weighing period. From these determinations and body weight data, food efficiency and average daily intake of test material were calculated.

Hematological examinations were conducted on blood obtained from the tails of 10 male and 10 female rats from each dietary level after 3, 6, 12, 18 and 24 months of continuous feeding. The examination consisted of a measure of erythrocyte count, hemoglobin, hematocrit, and total and differential leucocyte count. The following hematologic indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Urine analyses were conducted on specimens from the same animals that were used for the hematological examinations, at the same time intervals. Analyses included a measure of the 24-hour volume, concentration, and semi-quantitative tests for occult blood, sugar, protein, bilirubin and urobilinogen; color and appearance were noted; pH was measured. Sediment from pooled urine sampels were also examined microscopically. SAP, SGOT, SGPT, gamma-GTP, BUN, LDH and total protein were determined on blood samples taken from those rats used for hematologic and urinary analyses, at the same time intervals.

A record was kept of rats that died or were sacrificed *in extremis* during the study; tissues were preserved, whenever possible, for histopathologic evaluation.

After one year of continuous feeding, 10 rats from each group were sacrificed, by design, for gross and histopathologic evaluation and presented in the one-year interim report. After two years of continuous feeding, all surviving rats in all groups were sacrificed for gross and histopathological evaluation. Select organs (brain, heart, lungs, liver, spleen, kidneys, testes, stomach, thymus, adrenals and pituitary) were weighed at each sacrifice interval; organ weight/body weight ratios were calculated.

Tissues from each rat were examined grossly and preserved in 10% neutral buffered formalin and/or Bouin's solution. Tissues examined microscopically included: all gross lesions, thymus, spleen, lymph nodes, bone marrow, parathyroid bone, trachea, lung, heart, aorta, salivary gland, esophagus, stomach, small intestine,

cecum, colon, pancreas, liver, kidney, urinary bladder, pituitary, thyroid, adrenal, brain, spinal cord, peripheral nerve, eye, skin, mammary gland, testis, epididymis, prostate, uterus, ovary and skeletal muscle.

Appropriate statistical analyses were performed. Significance was judged at the 0.05 probability level in all cases except for weight gain analysis of variance where 0.05 and/or 0.01 probability levels were used.

Results:

The average body weight gains of male and female rats fed the highest dietary level (625 ppm) were significantly inferior to those of the controls and other test groups. After one month on test, the average body weight values for the high-level groups were 12% lower than the values for the respective controls.

With time, this difference lessened in the males and increased in the females. A slight reduction in weight gain was evident at 90 days in females fed 125 ppm. After 12 months of continuous feeding, males fed 625 ppm had average body weights 7% lower than control males; the high-level females had weights 22% lower than those of control females. After two years of continuous feeding, the average body weight gains of male and female rats fed 625 ppm were significantly inferior to those of the control and other test groups. At the termination of the study, males fed 625 ppm had average body weights 8% lower than control males and the high-level females had weights 34% lower than those of control females.

These male and female high-level rats consumed slightly less food and utilized their food for weight gain less effeciency than their respective controls or other test groups primarily during the first few months of the study. During the remainder of the first year and throughout the second year, there were no meaningful differences in food consumption and food utilization among control and test groups.

There were no clinical signs of toxicity that could be attributed to the feeding of Linu ron and no meaningful differences among mortality rates for controland treated rats.

During the two-year study, the average RBC count of males fed 50, 125 and 625 ppm was lower than the controls.

Female rats fed these levels has lower hematocrits and/or hemoglobin concentrations and RBC counts compared to controls.

The average RBC counts of the males fed 50 ppm was significantly lower than controls only at the 6-month examination and for males fed 125 ppm and 625 ppm at the 6 and 18-month examinations.

Females fed 50 ppm had a significantly lower hematocrit and hemoglobin at the 24-month examination; those fed 125 ppm had a lower RBC count at 6-months and lower hemoglobin at 12-months; those fed 625 ppm had a lower RBC count at 6-months, a lower hemoglobin at 6, 12, 18 and 24-months and a lower hematocrit at 12 and 18 months.

Regression analysis showed a good correlation between the increase of Linuron in the diet and the decrease of RBC counts of male rats and for the decrease of hemoglobin of female rats indicating a relationship between dose and hematologic response.

There was a definite relationship between RBC counts in the females, but only a slight relationship between dose and decrease in the hemoglobin and hematocrit of the males.

In addition to a decreased RBC count in male rats fed 125 or 625 ppm, these rats had significantly increased values for MCV and MCH with no change in MCHC. .

Male rats fed 50 ppm had a significantly increased value for MCH with no change in hemoglobin concentration or hematocrit.

These data strongly suggest reticulocytosis in male rats even though reticylocyte counts were not measured. All other hematological measurements were within the normal range, as established by the controls.

Female rats fed 625 ppm of test material tended to excrete a more dilute urine than controls as evidenced by an increased urine volume and decreased urine osmolality.

The results of all other urine analyses were essentially within the normal range, as established by the controls.

No statistically significant effects on SAP, SGPT, SGOT, gamma GTP and BUN levels were evident during the 24-month period that male or female rats were fed various levels of Linuron. LDH and total protein were either decreased and/or increased in a non-dose-related fashion.

The absolute and relative testicular weights of male rats fed 625 ppm and the absolute testicular weights of male fed 125 ppm were significantly increased, compared to controls. Male rats fed 625 ppm had significantly decreased absolute kidney and stomach weights. In addition, males fed 50 ppm or 625 ppm had significantly decreased absolute heart weights; however, the effects on heart weight were not dose-related.

The significance changes in absolute and/or relative testicular weights noted in male rats fed 125 or 625 ppm are consistent with the increased incidence of benign testicular interstitial cell adenomas observed in these groups.

The absolute weights of the heart, lungs, liver, kidneys, stomach and thymus were significantly decreased in female rats fed 625 ppm, compared to controls. Conversely, the relative weights of the brain, heart, lungs, liver, spleen, stomach and adrenals were significantly increased in these rats compared to controls. In addition, these female rats fed 625 ppm Linuron had a decreased relative thymus weight.

The absolute and relative organ weight changes observed in female rats fed 625 ppm Linuron are consistent with themarked decrease in final body weight and average body weight gain for this group of rats.

Compound-related histomorphologic changes were observed in the lymph-hematopoietic tissues (spleen, lymph nodes, thymus and bone marrow), epididymes, liver, kidneys, pancreas, uterus, testes, lungs, and thyroid glands.

Compound-related histomorphologic changes observed in hepatic tissues included hepatocellular megalocytosis and syncytium formation. The two dimentional area of affected hepatocytes was increased two to four times that of normal appearing hepatocytes in the same microscopic field.

Affected hepatocytes occasionally contained two or three nuclei with rare hepatocytes containing 12 to 15 nuclei.

In addition, fibroplasia was observed to radiate between hepatic cords. A significant increase ($p < 0.05$) in hepatic sinusoidal ectasia was observed in females fed 625 ppm. A significant increase ($p < 0.05$) in the incidence of focus/foci of hepatic cellular alteration was observed in male rats fed 125 or 625 ppm.

Lympho-hepatopoietic changes that were significantly increased ($p < 0.05$) included perivascular lymphocytic infiltration of hepatic periportal area in males fed 125 and 625 ppm; in the perivascular spaces of the kidneys in males fed 125 and 625 ppm; in the perivascular spaces of the lungs in males fed 625 ppm and in the interstitium of the pancreas in males fed 125 ppm and 625 ppm of Linuron.

An increase in the amount of hemosiderin deposited in the spleen, bone marrow and Kupffer cells was observed in males and females receiving 625 ppm. A similar increase in hemosiderin deposition was also observed in mesenteric lymph nodes of males fed 125 and 625 ppm.

Spleenic extramedullary hematopoiesis was significantly increased in high-dose female rats.

Females fed 625 ppm had increased incidences of thymic lymphoid atrophy and thyroidal cysts.

Significantly increased ($p < 0.05$) incidences of renal changes included tubular calculi in intermediate and high dose females, collecting duct ectasia in high dose females and mineralized formations and transitional cell hyperplasia of the renal pelvices ofmales fed 125 and 625 ppm.

An increased incidence of endometrial cystic hyperplasia was observed in females in the high dose group.

Significant increases ($p < 0.05$) in interstitial cell adenomas occurred in the testes of male rats receiving 125 and 625 ppm of Linuron.

	Control	50 ppm	125 ppm	625 ppm
Incidence	.057 (4/70)	.134 (9/67)	.271 (19/70)	.529 (37/70)
Fisher Exact p - value	-	.105	.004	< .0001
Mantel-Haenszel p - value	-	.20	.002	< .0001

A linear log-dose response relationship for all compound-treated groups was suggested by maximum likelihood probit analysis ($p < 0.001$); however the incidence of these benign tumors in rats receiving 50 ppm was not found to be significant at the 0.05 level of probability.

It should also be noted that chemically induced tumors in animals cannot with certainty be extrapolated directly to humans in regards to target organs or tumor type (benign vs. malignant).

Significant increases ($p < 0.05$) in the incidences of subacute perivasculitis/vasculitis of epididymal vessels were also observed in the 125 and 625 ppm males. All of the other changes reported were changes that were observed with similar frequency in both the control and treated rats.

Conclusion:

Significant increases in interstitial cell adenomas occurred in the testes of male rats receiving dietary concentrations of 125 and 625 ppm of Linuron. This IN effect triggers an oncogenic RPAR criterion. Other significant toxic effects occurred at 125 and 625 ppm in both sexes. Reticulocytosis was suggested in female and male rats at 50, 125 and 625 due to lower than normal RBC count and/or hemoglobin and hematocrit.

Classification: Core-Minimum Data

TOX/HED:th:WDYKSTRA:4-7-80

C. Trick 4/10/80